Plant diversity effects on soil multistability

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Abstract

Soil is the basis for life on Earth as we know it. Healthy and stable soil is a prerequisite for well-functioning terrestrial ecosystems and has, thus, been proposed to play a key role in plant diversity–ecosystem functioning relationships. The overall objective of this sub-project is to study multidimensional soil stability as affected by plant diversity in a long-term plant diversity experiment. We designed three coordinated work packages (WPs) to comprehensively assess soil multistability to environmental fluctuations and climate extremes by considering the biological, chemical and physical dimensions that are key for soil functioning. We will use all unique facilities and approaches of the Jena Experiment Research Unit by combining synthesis of long-term data in the *Main Experiment* and the *ΔBEF Experiment* with performing new soil analyses in the *DrY Experiment*, the *ResCUE Experiment* and a joint *CoMic Experiment*, to gain a better mechanistic understanding of plant diversity–ecosystem functioning relationships. In close collaboration with other sub-projects, we will assess biological, chemical and physical soil properties and stability indicators that will be used to calculate soil multifunctionality and multistability indices. In WP1, we will build on three unique datasets to explore short-term and long-term effects of plant diversity on the stability of soil (microbial) properties. In WP2, we will combine different datasets and approaches to explore if plant diversity effects on the magnitude and stability of soil properties increase with abiotic and biotic stresses. In WP3, we will combine measurements of the above-mentioned dimensions of soil stability to explore if plant diversity increases the stability of multiple soil properties under hot drought. This sub-project is at the heart of the Research Unit by testing the overarching hypotheses outlined in the Coordination Proposal of the Jena Experiment, contributing to all main experiments, sharing data and performing joint sampling campaigns with all sub-projects and, at the same time, introducing a novel concept of soil multistability as affected by plant diversity and climate extremes. We propose to use a combination of simple, high-throughput (e.g. bait-lamina test) and more sophisticated methods (e.g. extracellular polymeric substances analyses) to be able to investigate temporal dynamics of soil processes and their mechanistic basis. Taken together, the results of the three WPs will provide new insights into the stabilising

mechanisms of soil properties in the long term and in relation to climate extremes through plant diversity.

Keywords

biodiversity, climate extremes, ecosystem functioning, above-belowground interactions, multifunctionality, resistance, recovery

Background

State of the art

Soils are inherently complex and multidimensional, representing the melting pots of different spheres, the atmosphere, biosphere, lithosphere and hydrosphere. The interplay amongst the different ingredients (i.e. minerals, soil organic matter, living organisms, gas and water) causes soils to vary substantially in time and space (Kuzyakov and Blagodatskaya 2015, Eisenhauer et al. 2018a, Eisenhauer et al. 2021) creating habitats for soil biodiversity across multiple spatial scales (Bardgett and van der Putten 2014, F.A.O. et al. 2020). In fact, soils are one of the main global reservoirs of biodiversity, with more than 40% of living organisms in terrestrial ecosystems being associated with soils during their life cycle directly (Decaëns et al. 2006). Soil biodiversity provides many ecosystem functions (Bardgett and van der Putten 2014, Wall et al. 2015) that contribute to human well-being, including resources that support plant biomass production, such as nutrients and water, soil carbon storage and soil erosion control (Amundson et al. 2015, Wall et al. 2015). Consequently, concern about factors that influence soil ecosystem functioning in the context of climate change and biodiversity loss has attracted increasing scientific (Bardgett and van der Putten 2014, Amundson et al. 2015, Veresoglou et al. 2015) and public (Wall et al. 2015) attention.

Changes in plant diversity are key factors influencing soil organisms (Hooper et al. 2000, Scherber et al. 2010, Eisenhauer et al. 2013, Lange et al. 2015). Through bottom-up effects, diverse plant communities provide a higher quantity, quality and stable supply of plant-derived inputs to soil microorganisms and detritivores over time, as well as more structural diversity and stable microclimatic conditions (Hooper et al. 2000, Eisenhauer et al. 2013, Eisenhauer et al. 2017). This causes cascading effects on the abundance and diversity at higher trophic levels in the soil (Hooper et al. 2000, Scherber et al. 2010, Eisenhauer et al. 2013), which, in turn, change ecosystem functions mediated by soil organisms, such as litter decomposition (Vogel et al. 2013), soil carbon storage (Fornara and Tilman 2008, Lange et al. 2015, Lange et al. 2023) and soil aggregate stabilisation (Pérès et al. 2013, Gould et al. 2016). Given the ecological and economic importance of soil functions (Bardgett and van der Putten 2014, Amundson et al. 2015, Wall et al. 2015, F.A.O. et al. 2020), a key research priority is to understand whether maintaining high local plant diversity secures multiple soil-mediated functions under future environmental conditions (Eisenhauer et al. 2018a) and their stability (Strecker et al. 2016, Gottschall et al. 2021). Studies on relationships amongst plant diversity, soil organisms and functions indicate that the impact of biodiversity on the magnitude (Eisenhauer et al. 2010, Gottschall et al. 2021) and stability of soil processes (Huang et al. 2023) increases over their experimental duration. The slow build-up of plant community-specific and -representative soil communities is likely to have significant feedback effects on multiple ecosystem processes and, thus, on biodiversity-ecosystem functioning (BEF) relationships (Eisenhauer 2012Eisenhauer et al. 2019, Thakur et al. 2021, Eisenhauer 2012). More specifically, in the long-term, species-rich plant communities experience predominantly facilitative net effects by soil biota promoting plant community growth, such as decomposers, arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria, whereas species-poor plant communities are subject to antagonistic net soil effects due to the accumulation of pathogens (Eisenhauer 2012) with significant consequences for ecosystem functioning (Eisenhauer 2012, Eisenhauer et al. 2019, Thakur et al. 2021, Eisenhauer 2012).

The functioning of soil ecosystems is inherently multidimensional (Eisenhauer et al. 2018b). Measures to capture multifunctionality are increasingly being applied to summarise the capacity of an ecosystem to perform multiple functions (Eisenhauer et al. 2018b, Manning et al. 2018). Future environmental conditions may alter the relationship between biodiversity and ecosystem multifunctionality (Hector and Bagchi 2007, Lefcheck et al. 2015, Soliveres et al. 2016, Manning et al. 2018). Empirical evidence suggests that different plant species promote different ecosystem functions under different environmental contexts and global change scenarios (Isbell et al. 2011). Moreover, studies have highlighted that conserving local plant diversity is, therefore, a robust strategy to maintain multiple valuable (soil) ecosystem services under both present and future environmental conditions. Moreover, it is likely that higher plant diversity does not only enhance soil multifunctionality (Eisenhauer et al. 2018a, Schuldt et al. 2018), but also the stability of multiple soil functions as affected by environmental fluctuations and climate extremes.

Preliminary work

Work in SP4 in the first phase of FOR 5000. In SP4 of the first phase of this Research Unit, we designed three coordinated work packages (WPs) to test mechanisms driving potential shifts in activity periods above- and belowground. First, we proposed to test whether above- and belowground activity periods are linked to the effects of plant diversity on abiotic conditions (WP1). If so, are activity periods dependent upon longer-term effects of plant diversity on soils (i.e. soil history; WP2) or are activity periods more strongly associated with plant traits and history and their interaction with soil history (WP3)? In addition, we introduced WP0 to facilitate the PhD student's work in SP4 during the COVID-19 pandemic by starting a quantitative literature review of the research field (Asato et al. 2023).

Work package 0. We performed a systematic review of 94 studies, which reported 226 phenological observations, to evaluate current knowledge of soil microbial and animal phenology. Despite the increasing number of soil phenology reports, most research is still concentrated in/on few countries (centred in the Northern Hemisphere) and taxa (microbiota), with significant gaps in the most diverse regions of the globe (i.e. Tropics) and taxa (e.g. ants, termites and earthworms). In addition, biotic predictors (e.g. biodiversity and species interactions) have rarely been considered as possible drivers of soil organisms' phenology. Based on our results, we presented a guide on soil phenology research (Asato et al. 2023). Overall, we advocate that focusing on highly diverse ecosystems and key soil organisms, together with testing for direct and indirect effects of biodiversity loss and climatic stressors, is important for increasing our knowledge of soil functioning and predicting the effects of global change on terrestrial ecosystem functioning as a whole.

Work package 1. Here, we analysed the coupling of above- and belowground phenology in the *Main Experiment* in 2021 and found that it is partially driven by plant diversity (Fig. 2 in Asato et al. (2024)). As aboveground features, we measured plant height and greenness biweekly for one growing season. Belowground, we estimated root growth and soil detritivore feeding activity every two to three weeks for one full year. The activity of plant shoots peaked in the growing season, with peak plant height preceding peak photosynthetic rates, as indicated by the green-red index. Root growth started after leaf senescence and continued throughout the winter. Detritivores were active throughout the year, with two peaks in summer and high levels throughout the winter. Moreover, plant diversity increased the magnitude of plant height and soil activity throughout the year.

Moreover, we tested plant species richness effects on plant and soil phenology during leaf-flushing, leaf-senescence and over winter. The effects of plant diversity changed in magnitude and direction throughout the year, but mostly showed enhancing effects on individual activities, while the correlations between activity measures (indicating coupling, i.e. coinciding activity patterns) varied substantially amongst seasons and phenology indices (Figure 3 in Asato et al. (2024). Belowground activity was generally high and strongly driven by plant diversity, with positive plant diversity effects on the magnitude and stability of soil detritivore feeding activity. However, this was not synchronised with aboveground activity, leading to complex and opposite plant diversity effects on aboveground-belowground coupling that depended on the season.

Work packages 2 and 3 focused on soil and plant history as determinants of long-term plant diversity effects on the magnitude and phenology of soil detritivore feeding activity. While we performed all proposed measurements in the *ΔBEF Experiment* and in the *JenaTron Experiment* according to our initial proposal, the COVID-19 caused delay in sampling campaigns (samplings in the *Main Experiment* and *ΔBEF Experiment* were shifted from 2020 to 2021) and experiments (the *JenaTron Experiment* was postponed from 2021 to 2022). This resulted in significant delays in our data analyses. Therefore, the report on the objectives of WP2 and WP3 can only be based on results of the *ΔBEF Experiment*. We observed similar activity patterns across the treatments of the *ΔBEF Experiment* (Asato et al. unpubl.).

Total belowground activity increased significantly, activity variability decreased with plant species richness and activity duration was not significantly affected (Asato et al. unpubl.). This means that plant diversity increases the magnitude and stability of soil detritivore feeding activity. Importantly, the plant diversity effect was most pronounced in the treatment with plant and soil history and weakest in the treatment without plant and soil history. As hypothesised, this indicates that long-term plant diversity effects (i.e. with soil and plant history) on soil functioning are more pronounced than short-term effects (i.e. no history). While this has already been shown for the magnitude of soil functions before in the Jena Experiment (soil microbial biomass: Eisenhauer et al. (2010), Strecker et al. (2016); soil carbon concentrations: Lange et al. (2023)), this is the first time that we find stabilising effects of plant diversity on soil biological activity. A next important step would be to test if this stabilising effect on soil biological processes is caused by microclimate stabilisation (Huang et al. 2023), as soil temperature and moisture play key roles in determining biological activity.

Scientific expertise of the applicants and important work in preparation of the proposal. The applicants are soil ecologists with a strong history in the assessment of soil biodiversity and ecosystem functions in response to different environmental drivers, such as climate change (e.g.Eisenhauer et al. (2012), Cesarz et al. (2015), Thakur et al. (2017)) and plant diversity (e.g. Cesarz et al. (2013a), Eisenhauer et al. (2013), Eisenhauer et al. (2018a)). They have studied the temporal and spatial drivers of soil communities and ecosystem functions across ecosystems (e.g. Eisenhauer et al. (2011), Isbell et al. (2015), Guerrero-Ramírez et al. (2017), Craven et al. (2018), Doetterl et al. (2018), Gottschall et al. (2019), Gottschall et al. (2021), Smith et al. (2021), Kidinda et al. (2023)) and have linked soil communities to ecosystem functions (e.g. Lange et al. (2015), Schwarz et al. (2017), Beugnon et al. (2021)). The applicants use a wide range of methods to mechanistically understand the links between multiple soil taxa and processes (e.g. Scherber et al. (2010), Cesarz et al. (2013b), Eisenhauer et al. (2013)), nematodes as soil indicators (e.g. Eisenhauer et al. (2011), Cesarz et al. (2017)), soil microbial respiration and biomass (e.g. Eisenhauer et al. (2010), Strecker et al. (2016), Eisenhauer et al. (2018b), Cesarz et al. 2022), soil extracellular enzymes (e.g. Steinauer et al. (2015), Thakur et al. (2019), Kostin et al. (2021)). Furthermore, the applicants have expertise in different decomposition measurements (Vogel et al. 2013, Vogel et al. 2014, Thakur et al. 2017, Gottschall et al. 2019, Gottschall et al. 2021) and the analysis of soil aggregation (Pérès et al. 2013, Eisenhauer et al. 2018b) and extracellular polymeric substances (Zethof et al. 2020, Bettermann et al. 2021, Olagoke et al. 2022). As an expert in soil ecology, N. Eisenhauer has contributed to important biodiversity reports (F.A.O. et al. 2020, Pörtner et al. 2021).

Temporal and spatial stability. In previous work (Strecker et al. 2016), we tracked soil microbial basal respiration and biomass over a time period of 12 years and examined the role of plant diversity and functional group composition for the spatial and temporal stability of soil microbial properties (Fig. 3 in Strecker et al. (2016)). We found that: 1) plant species richness consistently increased soil microbial properties after a time lag of four years since the establishment, 2) plant species richness had minor effects on the spatial stability of soil microbial properties, whereas 3) the functional composition of plant communities significantly affected spatial stability of soil microbial properties and 4) the effect of plant diversity on the temporal stability of soil microbial properties turned from negative to neutral, suggesting that the recovery of soil microbial communities from former arable land-use takes more than a decade. These results highlight the importance of plant diversity and functional group composition for the spatial and temporal stability of soil microbial properties and call for longer-term studies exploring plant diversity–soil stability relationships. There is increasing evidence that spatial and temporal dynamics of biodiversity and ecosystem functions play an essential role in BEF relationships. In a recent collaborative work (Gottschall et al. 2021), we propose a novel conceptual framework integrating spatiotemporal dynamics in BEF relationships and hypothesised a positive plant (in this case: tree) species richness effect on soil ecosystem functions through the spatial and temporal stability of biotic and abiotic soil properties, based on species complementarity and asynchrony. The study revealed that overall soil microbial biomass along a tree diversity gradient was partly explained by: (1) enhanced temporal stability of soil surface temperature and (2) decreased spatial stability of soil microbial biomass. Overall, spatial stability of soil microbial properties was positively correlated with their temporal stability. However, it remains unclear if and how vegetation-induced differences in soil microbial communities contribute to this stability itself, for example, to abiotic (drought tolerance) and biotic stress (protection against plant antagonists) by specific microbial functionalities, such as the production of extracellular polymeric substances (EPS). This is an important research frontier, given that spatiotemporal dynamics may indeed be crucial determinants in BEF relationships and highlight the importance of vegetation-induced microclimatic conditions for the stable provisioning of soil ecosystem functions and services.

Stability to drought. In the *Drought Experiment* in the context of the Jena Experiment, we studied litter mass loss rates and soil microbial properties in response to plant species richness and summer drought. Decreasing plant diversity and summer drought decreased litter mass loss rates and soil microbial properties (Vogel et al. 2013). However, we found no interactive effects between plant diversity and drought, suggesting that, in young biodiversity experiments (i.e. seven years after establishment of the experiment), plant diversity loss and summer drought may independently impede soil processes. Moreover, decomposer communities may be highly adapted to decomposing plant community-specific litter material, even in situations of environmental stress (Vogel et al. 2013). An enhanced soil temperature stability (i.e. the climate buffering effect of plant diversity; Huang et al. (2023)), as well as temporarily more stable soil detritivore activity rates at high plant diversity (Asato et al. unpubl.) indicate that long-term studies are urgently needed to assess the potential of plant diversity to stabilise soil processes. Additionally, short-term studies are necessary on drought resistance and recovery indicated through specific microbial traits (e.g. EPS production) fostered by plant diversity. Overall, combined effects of plant diversity and drought on soil ecosystem functions remain poorly explored, especially with regard to their short- and long-term effects.

Soil aggregate stability is known to depend on plant community properties, as they influence the temporary and transient binding agents, such as decomposed organic matter, fine roots, fungal hyphae and extracellular polymeric substances (EPS). However, little is known about the relative importance of these drivers and the role of soil organisms in mediating plant community effects. Pérès et al. (2013) found that soil aggregate stability increased significantly from monocultures to plant species mixtures. Using structural equation modelling, plant community effects could be explained by variations in root biomass, soil microbial biomass, soil organic carbon concentrations (all positive relationships, Figure 2c in Pérès et al. (2013)) and earthworm biomass (negative relationship). Given that the positive effects of plant diversity on root biomass (Ravenek et al. 2014), soil microbial biomass Eisenhauer et al. 2010, Strecker et al. 2016) and soil organic carbon concentrations (Lange et al. 2015, Lange et al. 2023) have increased considerably over time, we expect long-term plant diversity effects on soil aggregate stability to be stronger in the meantime. Moreover, results by Gould et al. (2016) suggest that the consideration of root traits is important to mechanistically understand plant diversity effects. We are thus planning to collaborate with SP03 (The Jena Experiment: SP03 2024). Next to that, the role of EPS for plant diversity-soil aggregate stability relationships has not been explored, especially in connection to stress (abiotic and biotic) and potential plant resistance, but they are likely to contribute key mechanistic insights (Costa et al. 2018).

In the history of BEF research, we have seen an evolution of topics and important transitions (Hines et al. 2019). Here, we propose to take another significant step forward by exploring the significance of plant diversity for soil processes under different climatic contexts and exploring the buffering effects of plant diversity on soil multistability by acknowledging the complexity and multidimensionality of soil. Given the preliminary work above, we have convincing empirical evidence to expect that biological, chemical and physical dimensions of soil stability should increase with plant diversity and that plant diversity increases soil multistability under climate extreme events.

Objectives and work programme

Anticipated total duration of the project

Four years (2024-2027).

Objectives

The overall objective of this sub-project is to study multidimensional soil stability, including temporal stability, resistance and recovery as defined in the Coordination Proposal, as affected by plant diversity. We designed three coordinated work packages (WPs) to comprehensively assess soil multistability by considering biological, chemical and physical dimensions that are key for soil functioning. We propose to use all unique facilities of this Research Unit by combining synthesis of long-term data in the *Main*

Experiment and the *ΔBEF Experiment*, with performing new soil analyses in the *DrY Experiment*, *ResCUE Experiment* and a joint *CoMic Experiment*. In close collaboration with other sub-projects, we will assess soil processes and stability indicators that will be used to calculate soil multifunctionality and multistability indices. In WP1, we will explore short- and long-term effects of plant diversity on the stability of soil (microbial) properties. In WP2, we will explore if plant diversity effects on the magnitude and stability of soil properties increase with abiotic and biotic stresses. In WP3, we will explore if plant diversity increases the stability of multiple soil properties under hot drought. Taken together, results of these three WPs will provide novel insights into the stabiliing mechanisms of soil properties along plant diversity gradients. More specifically, we test the following hypotheses: *Hypotheses of WP1* (*Fig. 1*)*: Soil temporal stability*. **H1.1:** The relationships between soil microbial properties (respiration and biomass C) and plant diversity are positive and strengthen over time. **H1.2:** Plant diversity decreases the temporal stability of soil microbial properties in the short term (2003-2009), does not significantly affect it in the intermediate term (2010-2016) and increases it the long term (2017-2024) of the experiment (Strecker et al. 2016). **H1.3:** The stabilising effects of plant diversity on soil microbial properties as affected by (repeated) summer drought(s) (2018 and 2019) are more pronounced in sub-plots with plant and soil history (representing long-term effects) than in sub-plots without plant and soil history (representing short-term effects). **H1.4:** Positive plant diversity effects on the temporal stability of soil microbial properties can be better predicted, based on resistance to than on the recovery from drought. *Hypotheses of WP2* (Fig. 2)*: Soil stability along stress gradients.* Plant diversity effects on the magnitude and stability of soil microbial properties increase with increasing levels of stress, such as indicated by years with a high drought index (**H2.1**; Huang et al. (2023)) and increasing drought severity (**H2.2**). Moreover, plant communities stressed with the presence of plant pathogens will show stronger stabilising effects of plant diversity on soil properties than plant communities without plant pathogens (**H2.3**;The Jena Experiment: SP08 (2024)). *Hypotheses of WP3* (Fig. 3): Soil stability under hot drought. **H3.1**: Plant diversity increases the temporal stability, as well as resistance to and recovery after a hot drought, of multiple soil properties. **H3.2**: Positive plant diversity effects on the temporal stability of soil microbial properties can be better predicted, based on resistance to than on the recovery from a hot drought.

Work programme including proposed research methods

All PIs of this sub-project will contribute to all WPs, while each PI will be responsible for the supervision of certain methods, according to their main expertise (indicated in “Research methods” below). We propose to integrate different dimensions of soil stability by assessing biological (soil microbial respiration, soil microbial biomass, soil extracellular enzyme activities, soil detritivore feeding activity), chemical (SOM thermal stability) and physical processes (soil aggregate stability), soil microclimate (soil temperature and soil moisture; SPZ1), as well as their stability. Therefore, we will calculate soil ecosystem multifunctionality and -multistability and explain expected positive plant diversity effects mechanistically by including data on, for example, EPS (this SP), root traits (The Jena Experiment: SP03 2024), soil carbon and nutrient dynamics (The Jena Experiment: SP04 2024, The Jena Experiment: SP05 2024), soil microbial community composition and drought-tolerance traits (The Jena Experiment: SP08 2024, The Jena Experiment: SP09 2024, The Jena Experiment: SP11 2024) and root biomass (The Jena Experiment: SPZ1 2024). SP07 will contribute to all main experiments, share data and perform joint sampling campaigns with all sub-projects and, at the same time, introduce a novel concept of soil multistability as affected by plant diversity and climate extremes. We propose to use a combination of simple, high-throughput (e.g. bait-lamina test; Eisenhauer et al. (2014)) and more sophisticated methods (e.g. EPS analysis) to be able to investigate the temporal dynamics of soil processes (e.g. Thakur et al. (2017), Asato et al. unpubl.) and their mechanistic basis. The focal soil processes were carefully chosen to represent different dimensions of soil stability. Plant root biomass is an indicator of belowground primary production and is often related to soil carbon storage (Lange et al. 2015) and soil aggregation (Gyssels et al. 2016). While soil microbial respiration indicates microbial decomposition activity (Lange et al. 2015, Eisenhauer et al. 2018b), soil microbial biomass is a proxy for belowground secondary production, soil enzyme and phosphorus dynamics (Hacker et al. 2015), soil nitrogen leaching (Leimer et al. 2016) and both variables are powerful predictors for nutrient availability and soil carbon storage (Lange et al. 2015, Thakur et al. 2015). Aggregate stability is an important soil physical indicator relevant for soil erosion control and sustainability (Kamamia et al. 2021). Soil detritivore feeding activity is a sensitive indicator of soil biological activity as affected by environmental change (Thakur et al. 2015) and of the energy flux to soil detritivores (Schwarz et al. 2017; The Jena Experiment: SP11 2024). Soil extracellular enzyme activities are highly sensitive indicators of nutrient dynamics mediated by soils (e.g. Steinauer et al. (2015), Kostin et al. (2021)). Measurements of EPS will help us to mechanistically explain temporal variations in soil aggregate stability as a transient binding agent (Bettermann et al. 2021, Olagoke et al. 2022) and will link our process measures to soil microbial communities and their response to biotic and abiotic stress (Vuko et al. 2020, The Jena Experiment: SP08 2024, The Jena Experiment: SP09 2024). We expect that aggregate stability increases due to the induction of EPS production as an important drought stress trait (at least under medium drought intensity levels).

WP1: Soil temporal stability (Main Experiment and ΔBEF Experiment)

This WP will build on three unique datasets to explore **short-term and long-term effects of plant diversity on the stability of soil (microbial) properties**. First, we will analyse the long-term dataset of soil microbial properties (soil basal respiration, soil microbial biomass and soil microbial respiratory quotient) that we have assembled since the beginning of the *Main Experiment* (Fig. 1A), with yearly measurements during peak plant biomass (late May/early June) from 2003-2022 (we will perform additional sampling campaigns in 2023 and 2024 to complete the dataset; Fig. 1B) (Eisenhauer et al. 2010, Strecker et al. 2016). We will use moving-window analysis of detrended data (Wagg et al. 2022) to test the hypothesis that plant diversity effects on the magnitude and stability of soil microbial properties increase over time (**H1.1** and **H1.2**; (Fig. 1C). Second, we will use data that we have assembled in all sub-plots of the *ΔBEF Experiment* from

2017-2022 to directly compare short- and long-term plant diversity effects on the magnitude and stability of soil microbial properties as affected by summer drought event(s) in 2018 and 2019. We will follow the approach by P. Dietrich et al. (unpubl.) to determine drought resistance (2018 vs. 2017 and 2019 vs. 2017) and recovery (2020 vs. 2018 and 2020 vs. 2019), as well as temporal stability (CV-1) of soil microbial properties. Again, we expect to see that plant diversity–ecosystem functioning and –stability relationships will be stronger in sub-plots representing long-term effects (with plant and soil history) as compared to those representing short-term effects (without plant and soil history; **H1.3**; Vogel et al. (2019)). Moreover, we expect that temporal stability of soil microbial properties can be better predicted, based on short-term resistance than on recovery (**H1.4**; Fig. 1D; F. Isbell et al. unpubl.). Third, we will investigate the seasonal dynamics of soil microbial properties (soil basal respiration, soil microbial biomass and soil microbial respiratory quotient [all 80 plots]); soil enzymes (in the C, N and P cycle [44 selected plots]), soil detritivore feedings activity (bait-lamina strips [44 plots]), soil microclimate stability (temporal stability of soil temperature and moisture [all 80 plots]), soil aggregate stability (water-stable aggregates [44 plots]) and EPS [44 plots] at four different time points in the *Main Experiment* (following the time steps of SP11). Such information is critical to assess if plant diversity enhances soil multifunctionality and multistability by enhancing the stability of environmental conditions across seasons (Huang et al. 2023). Moreover, we will use the main sampling campaign at peak plant biomass to determine the SOM thermal stability (combustion [all 80 plots]). These biological, chemical and physical soil stability indicators will be used to calculate soil multistability indices. We will have to perform subset analyses for data measured on 80 and 44 plots (1, 4 and 16 plant species plots, as done in the *ResCUE Experiment*), respectively, to test the hypothesis that plant diversity increases soil multistability (**H1.2**; Huang et al. (2023)).

Collaboration: All sampling campaigns will be conducted in close collaboration with other sub-projects to facilitate data exchange and maximise comparability (e.g. performing measurements on the same samples. All our data will be made accessible to any collaboration effort. For instance, soil microbial biomass data will be provided to SP04, SP05, SP08, SP11 and SPZ2 (The Jena Experiment: SPZ2 2024) (WP3). We plan to combine data on EPS and soil enzymes (this SP) with data on soil microorganisms producing EPS and other drought stress response traits as well as being involved in N and P turnover (SP08). Root trait data by SP03 will be considered as an explanatory variable for soil aggregate stability. PLFA data measured by SP11 will be used as explanatory variables to test the idea that plant diversity increases the proportion of fungal biomass as compared to bacterial biomass (Eisenhauer et al. 2017) and that a higher proportion of fungi increases the stability of soil processes (Kostin et al. 2021). Soil temperature and moisture data by SPZ1 will be used to include soil microclimatic conditions. **Analyses:** We will use mixed-effects models and moving-window analyses to test effects of plant diversity and time (or subplot treatment in the *ΔBEF Experiment*) and their interaction on the magnitude and stability of soil biological, chemical and physical properties, as done previously (Eisenhauer et al. 2010, Strecker et al. 2016) to test our hypotheses.

WP2: Soil stability along stress gradient (Main Experiment, DrY Experiment and CoMic Experiment)

In this WP, we will again combine different datasets and approaches to explore **if plant diversity effects on the magnitude and stability of soil properties increase with abiotic and biotic stresses**. First, we will analyse the long-term dataset of soil microbial properties (see above; WP1) in the *Main Experiment* (Fig. 2A) to test plant diversity effects on the mean and temporal stability (moving window). Extending analyses in WP1, effect sizes of plant diversity effects will then be correlated with different climatic datasets (climate conditions and climate extremes) in each of the years. Therefore, climate data will be based on different durations prior to the sampling event. Second, in the *DrY Experiment* (Fig. 2B) we will take measurements of soil microbial properties (soil basal respiration, soil microbial biomass and soil microbial respiratory quotient; all pots for resistance and recovery), soil enzymes (all pots), soil aggregate stability (selected pots; only resistance due to feasibility) and EPS (selected pots; only resistance). The non-drought (100% precipitation) treatment will serve as control for resistance calculations. Comparisons between measurements shortly after drought and 6 weeks after drought will be used to calculate recovery after drought. Taken together, these data will allow us to test the shape of response of plant diversity effects on the resistance and recovery of soil properties. We expect that plant diversity effects on the magnitude and stability of soil microbial properties to increase with increasing levels of stress, such as indicated by years with a high drought index or increasing drought severity (**H2.1** and **H2.2**; Huang et al. (2023), Fig. 2C and Fig. 2D). Third, we will contribute to the *CoMic Experiment* (*MockCoMic*) run by SP08 to test abiotic and biotic stressors on the magnitude and stability of soil properties. More specifically, we will take measurements of soil microbial properties (soil basal respiration, soil microbial biomass and soil microbial respiratory quotient), soil aggregate stability and EPS in 80 selected pots (2 water regimes x 2 sampling points (drought and recovery) x 2 microbial Mock communities (EPS and spore-forming communities as important microbial traits for drought resistance) x 2 Oomycete treatments (with and without) at late drought (one time point) in five independent replicates). This will be done to test if certain microbial traits (EPS production and spore formation) improve plant resistance or resilience towards drought and biotic stress (pathogens) as potential stabilising effects for soil properties. This will help us to get a better mechanistic understanding about the role of certain microbial traits under abiotic and biotic stress for the potential stronger stabilising effects of plant diversity on soil properties (**H2.3**). We will contribute our data to a joint paper led by the PhD student in SP08. **Collaboration:** All sampling campaigns will be conducted in close collaboration with other SPs, as described above. Data on soil microbial biomass and EPS will be provided to SP08 for a joint publication. All data will be made accessible to any collaboration effort. Root trait data by SP03 will be considered as an explanatory variable for soil aggregate stability. Soil temperature and soil moisture data by SPZ1 will be used. **Analyses:** We will use mixed-effects models (and moving-window analyses; for *Main Experiment*) to test the effects of plant diversity, time (only for *Main Experiment*), stress severity and their interaction on the magnitude and stability (temporal stability, resistance and recovery) of soil biological, chemical and physical properties to test our hypotheses.

WP3: Soil stability under hot drought (ResCUE Experiment)

In this WP, we will combine measurements of different dimensions of soil stability to explore ***if plant diversity increases the stability of multiple soil properties under hot drought***. In the *ResCUE Experiment* (Fig. 3A), we will perform repeated measurements of soil microbial properties (soil basal respiration, soil microbial biomass and soil microbial respiratory quotient [3 times]); soil enzymes (in the C, N and P cycle [3 times]), soil detritivore feedings activity (bait-lamina strips [6 times, every 2 weeks]), soil aggregate stability (water-stable aggregates [3 times]) and EPS (1 time) in all lysimeters (Fig. 3B). These data will be used to test the hypothesis that plant diversity increases the temporal stability, as well as resistance to and recovery after a hot drought, of multiple soil properties and multistability (**H3.1**, Fig. 3C) and that positive plant diversity effects on the temporal stability of soil microbial properties can be better predicted, based on resistance to than on the recovery from a hot drought (**H3.2**). **Collaboration:** All sampling campaigns will be conducted in close collaboration with other SPs, as described above. All data will be made accessible to any collaboration effort. Soil microbial biomass data will be provided to SP04, SP05, SP08 and SP11. Root trait data by SP03 will be considered as an explanatory variable for soil aggregate stability. Again, PLFA data measured by SP11 will be used as explanatory variables to test the idea that plant diversity increases the proportion of fungal biomass as compared to bacterial biomass (Eisenhauer et al. 2017). Soil temperature and soil moisture data by SPZ1 will be used. **Analyses:** We will use mixed-effects models to test effects of plant diversity on the magnitude and stability (temporal stability, resistance and recovery) of soil biological, chemical and physical properties.

Research methods

For all measurements, we will take multiple soil cores per plot (5 cm in diameter) or pot (2 cm in diameter) to 10 cm depth. We will combine samples per plot/pot in one bulk sample, sieve at 2 mm (to remove any large roots or animals) and store it at 4°C until measurement. In all experiments, we will conduct all sampling campaigns in close collaboration with other subprojects and share samples for subsequent analyses. Subsamples will be taken for the different measurements outlined below. For a rough time plan, see Fig. 4.

Soil microbial respiration, soil microbial biomass and soil microbial respiratory quotient (*supervised by Nico Eisenhauer*). Soil microbial biomass (µg C g-1 soil dw) and basal respiration (µl O_2 h-1 g-1 soil dry weight) will be measured using an O_2-microcompensation apparatus (Scheu 1992), as done before (Eisenhauer et al. 2010). The soil microbial respiratory quotient (metabolic oxygen quotient, qO_2; µl O_2 mg Cmic-1 h-1) will be calculated as a measure of the metabolic efficiency of the microbial community by dividing soil microbial basal respiration by soil microbial biomass (Eisenhauer et al. 2013).

Soil detritivore feeding activity (*supervised by Nico Eisenhauer*) will be assessed using the bait lamina test as a commonly used rapid ecosystem function assessment method (Eisenhauer et al. 2014, Thakur et al. 2017). Sticks will be filled with an artificial organic bait substrate and inserted vertically into the soil with the uppermost hole just beneath the soil surface. Five (*Main Experiment*) or four strips (*ResCUE Experiment*) placed at a distance of approximately 20 cm will be used per plot to account for spatial heterogeneity. After three or four weeks of exposure, the bait lamina strips will be removed from the soil, directly evaluated in the field and replaced by a new bait strip. For each plot, the mean bait consumption will be calculated.

Soil (extracellular) enzymes (*supervised by Simone Cesarz*) will be measured to gain insights into dynamic responses of soil microbial community functioning. The selected enzymes are β-D-1,4-glucosidase, β-1,4-N-acetyl-glucosaminidase, β-1,4-xylosidase and acid and alkaline phosphatase. These are hydrolytic enzymes commonly used to assess changes in activities involved in the carbon, nitrogen and phosphorus cycles (German et al. 2011, Thakur et al. 2019, Kostin et al. 2021). Soil enzyme activities will be measured using a fluorimetric method based on the release of methylumbelliferone (MU) from MU-labelled substrates adapted from Saiya-Cork et al. (2002). Enzyme activities will be measured with a microplate reader using end-point measurements (BMG Labtech, Ortenberg, Germany) at 365 nm excitation and 450 nm emission (Thakur et al. 2019, Kostin et al. 2021). The end-point for each enzyme will be determined by saturation curves (when enzyme activity levels off), based on the optimal substrate concentration, buffer solution and its pH. We will calculate and consider mass-specific enzyme activity (MSEA), which is the extracellular enzyme activity per unit of microbial biomass, to determine if the changes in the extracellular enzyme activity are caused by changes of soil microbial biomass (Steinweg et al. 2013).

Soil aggregate stability (*supervised by Nico Eisenhauer*) will determine the resistance of soil aggregates against water as a disintegrating force. We will apply an approach modified from Kemper and Rosenau (1986). The resulting index represents the percentage of water-stable macroaggregates (> 250 µm). The re-wetting and agitation of the tested soil aggregates causes the compression of entrapped air inside of them resulting in a process called slaking, which is a function of re-wetting intensity, volume of entrapped air and aggregate shear-strength (Le Bissonnais 1996). Stable aggregates will be sand-corrected (Rieke et al. 2022). Additionally, debris (i.e. coarse matter) will be separated from the water-stable fraction to correctly determine the water-stable aggregates (WSA) fraction of the sample: %WSA = (water stable fraction-coarse matter)/ (4 g-coarse matter).

Extracellular polymeric substances (**EPS**, *supervised by Cordula Vogel*). To test the role of EPS contents for soil aggregation and plant resistance under stress (abiotic and biotic) as influenced by plant diversity, we will apply the EPS extraction method using cation exchange resin as proposed for soil (Redmile-Gordon et al. 2014, Bettermann et al. 2021). Briefly, fresh sieved (2 mm) soil will be extracted with calcium chloride ($CaCl_2$) solution (0.01 M) to remove soluble microbial products. Afterwards, EPS will be extracted using cation exchange resin as described in Zethof et al. (2020). EPS-polysaccharide content will be quantified using the method of Dubois et al. (1956) and EPS-protein using a Lowry assay as described by Redmile-Gordon et al. (2013). Additionally, specific EPS-protein and EPS-polysaccharide concentrations will be calculated by normalising EPS per unit soil to the unit of soil microbial biomass used to indicate EPS production efficiency. The EPS production efficiency will give us closer insight into the microbial investment under stress.

SOM thermal stability (*supervised by collaborator Luiz A. Domeignoz-Horta*). We will use ramped thermal rock-eval® pyrolysis (RE) to evaluate SOM quantity and quality (Soucémarianadin et al. 2018), as done by the collaborator before (Domeignoz-Horta et al. 2021, Domeignoz-Horta et al. 2023). Soils will be pyrolysed over a temperature ramp from 200 to 650°C, followed by combustion to 850°C using a rock-eval 6 pyrolyser (Vinci Technologies) at the Institute of Earth Sciences of the University of Lausanne (Switzerland). Hydrocarbons released during this process will be measured by a flame ionisation detector. The resultant thermogram will be used to calculate the I index ("labile carbon fraction") and R index ("recalcitrant carbon fraction") as previously (Sebag et al. 2016). It was recently shown that the SOM thermal signature has an effect on mass-specific respiration and mass-specific enzymatic activity (Domeignoz-Horta et al. 2021). Thus, this measurement can be used to link the influence of plant diversity on soil carbon pools and the consequences for microbial activity.

Soil multifunctionality and multistability (*supervised by Nico Eisenhauer*). The data will be analysed for multifunctionality according to Byrnes et al. (2014) using the averaging and the multiple thresholds approach (e.g. Eisenhauer et al. (2018a)). We will extend this framework from multifunctionality to multistability, a novel concept that has only been developed recently in ecology (work in progress by Jena Experiment Humboldt Fellow Dr. Guiyao Zhou, as well as collaborator Prof. Dr. Takehiro Sasaki). Briefly, to calculate average soil multifunctionality and multistability, we will standardise all soil properties and the stability of these soil properties to values ranging between 0 and 1 and then calculate the average level of soil multifunctionality and the average level of soil multistability per plot/pot as the mean of the standardised soil processes. We are aware of the advantages and disadvantages of presenting aggregate measures of soil multifunctionality and multistability (for review see Manning et al. (2018)), which is why we put equal emphasis on the results based on individual measures of the magnitude and stability of soil processes and show results of the multiple thresholds approach. To evaluate whether multiple soil processes and their stability are simultaneously performing at high levels, we will create an index of the number of functions surpassing different thresholds in each experimental plot/pot. This threshold reflects the percentage of the maximum observed value of each soil process or its stability (Byrnes et al. 2014).

Handling of research data

All data will be deposited in and published through the Jena Experiment Database, according to the Jena Experiment data policy.

Relevance of sex, gender and/or diversity

This sub-project is led by two female and one male PI. Next to the official advertisement that includes statements like "Women are expressly invited to apply; the same applies to people with disabilities", we will actively contact collaborators and ask to forward our advertisement to promising female master students. We fully embrace diversity and believe that this is the key to successful teams. As a consequence, the Experimental Interaction Group (where the PhD student will be hosted) is highly diverse in terms of gender, cultural background and expertise (currently ~ 62% females amongst postdocs, PhD students and technicians; from 10 nationalities).

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Hosting institution

German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig

Conflicts of interest

The authors have declared that no competing interests exist.

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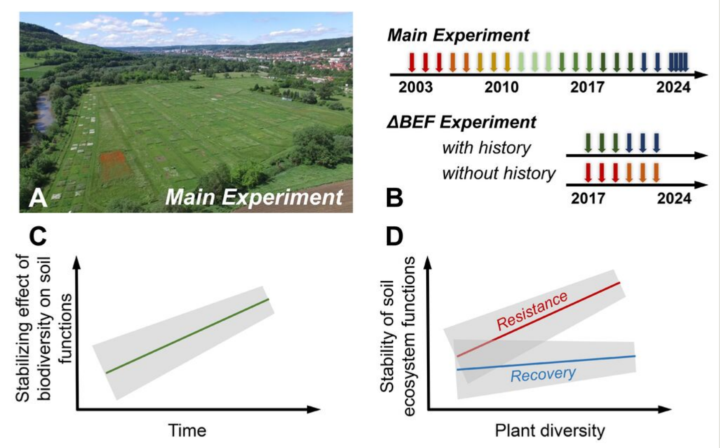


Figure 1.

Figure depicting the main data sources, analyses and hypotheses of Work Package (WP) 1. A: The field site of the Main Experiment in the Jena Experiment. B: Tentative timepoints of sampling campaigns. This WP will build on three unique datasets to explore short-term and long-term effects of plant diversity on the stability of soil (microbial) properties in the Main Experiment (yearly measurements of soil microbial properties; 2003-2024), ΔBEF Experiment (yearly measurements of soil microbial properties; 2017-2023) and seasonal measurements of focal soil processes in plots of the Main Experiment in 2024. Illustration of the hypotheses (C) that plant diversity effects on the magnitude and stability of soil microbial properties increase over time and (D) that the temporal stability of soil microbial properties can be better predicted, based on short-term resistance than on recovery.

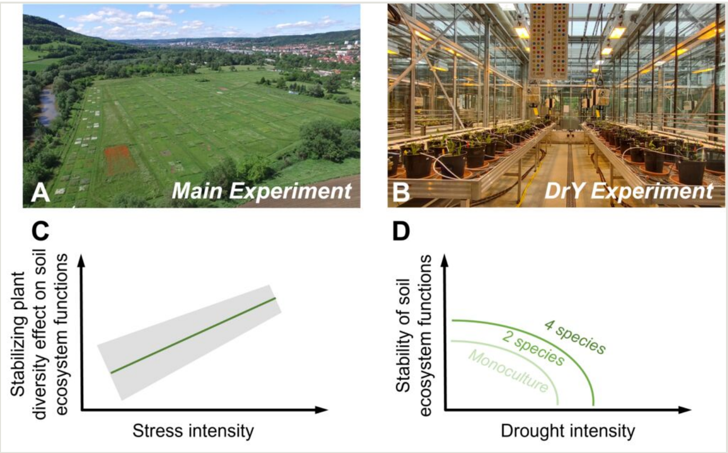


Figure 2.

Figure depicting the main data sources, analyses and hypotheses of Work Package (WP) 2. A: The site at which the Main Experiment of the Jena Experiment is located. B: Photo of the DrY Experiment. Illustration of the hypotheses (C) that plant diversity effects on the magnitude and stability of soil microbial properties increase with increasing levels of stress and (D) that the stabilising effects of plant diversity increase with increasing drought intensity, until a certain threshold is reached.

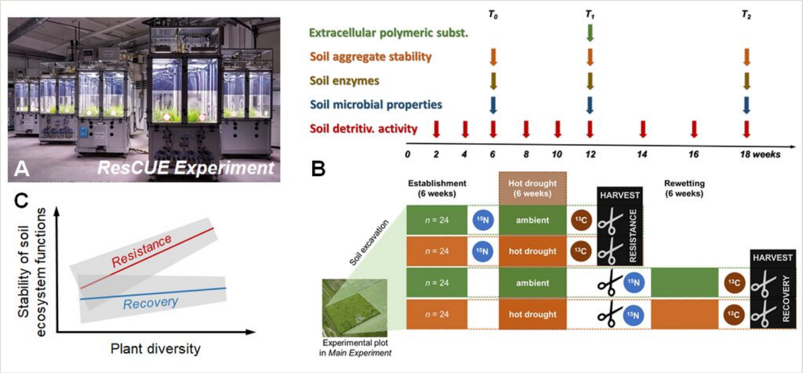
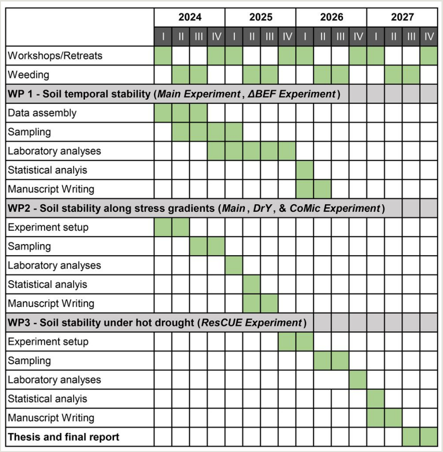


Figure 3.

Figure depicting the data sources, analyses and hypotheses of Work Package (WP) 3: A: Experimental units used for the ResCUE Experiment in the iDiv Ecotron. B: The main sampling campaigns of the ResCUE Experiment. We will measure different biological, chemical and physical soil processes repeatedly over the course of the experiment. While soil detritivore feeding activity will be measured every two weeks (8 campaigns x n = 96 lysimeters), soil microbial properties, soil extracellular enzymes and soil aggregate stability will be measured three times (right before the experimental drought [Time0 = T0; n = 96]; right after the drought [T1; n = 96]; and after 6 weeks of recovery [T2; n = 48]) and exopolysaccharides will be measured once (T1; n = 96). C: Illustration of the hypothesis that plant diversity increases the temporal stability, as well as resistance to and recovery after a hot drought, of multiple soil properties and multistability.



	2024				2025				2026				2027			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Workshops/Retreats	🟩			🟩	🟩			🟩	🟩			🟩	🟩			🟩
Weeding		🟩	🟩			🟩	🟩			🟩	🟩			🟩	🟩	
WP 1 - Soil temporal stability (*Main Experiment* , *ΔBEF Experiment*)																
Data assembly	🟩	🟩	🟩													
Sampling		🟩	🟩	🟩	🟩											
Laboratory analyses				🟩	🟩	🟩	🟩	🟩								
Statistical analyis									🟩							
Manuscript Writing									🟩	🟩						
WP2 - Soil stability along stress gradients (*Main* , *DrY* , & *CoMic Experiment*)																
Experiment setup	🟩	🟩														
Sampling			🟩	🟩												
Laboratory analyses					🟩											
Statistical analyis						🟩										
Manuscript Writing						🟩	🟩									
WP3 - Soil stability under hot drought (*ResCUE Experiment*)																
Experiment setup								🟩	🟩							
Sampling										🟩	🟩					
Laboratory analyses												🟩				
Statistical analyis													🟩			
Manuscript Writing													🟩	🟩		
Thesis and final report															🟩	🟩

Figure 4.

Timetable of the work programme.